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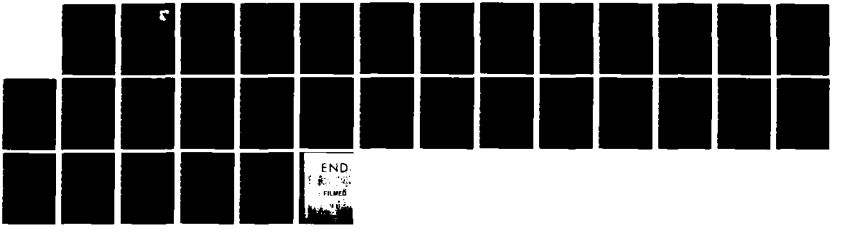

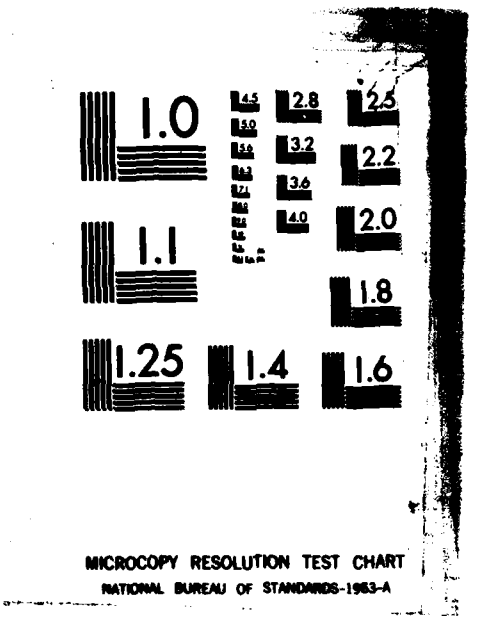

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BONE MINERAL ANALYSIS OF RAT VERTEBRAE FOLLOWING SPACE FLIGHT: COSMOS 1129

E. PAUL FRANCE
CLARENCE M. OLOFF
LEON E. KAZARIAN

AUGUST 1983

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AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-83-055

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

HENNING E. VON GIERKE, Dr Ing
Director
Biodynamics and Bioengineering Division
Air Force Aerospace Medical Research Laboratory

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The mission of COSMOS 1129 was to investigate the effects of 18.5 days of microgravity and readaptation to earth's gravity for various organisms. This study describes comparative mineral/element content of vertebral centra for rats flown aboard COSMOS 1129 (F) and rats from a ground based synchronous control study (S). F and S rats were sacrificed on a predetermined readaptive schedule following actual or simulated spaceflight recovery (R) at R+0, R+6, and R+29 days. 947 cleaned individual vertebral centra were harvested from these animals and stored frozen in sterile distilled water to await analysis. In preparation for mineral/

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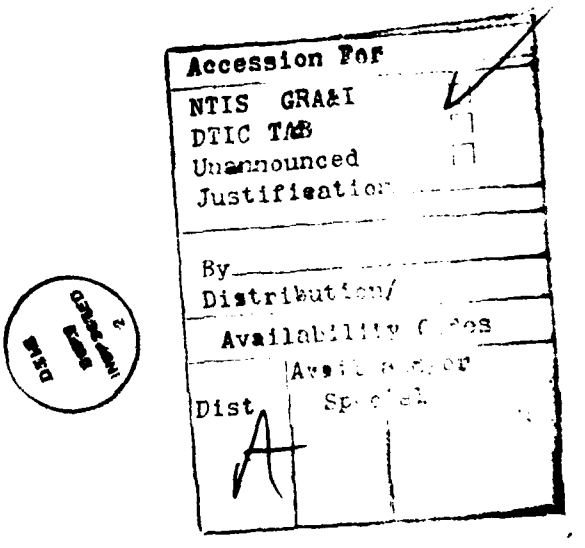
element analysis each specimen was dried, weighed and digested in nitric acid. The prepared samples were analyzed for Ca^{2+}, PO_4^{-}, K, Na, Ba, Sr, Fl, Mg, Pb, Mn, and Y using either atomic absorption spectrophotometry or colorimetric analysis. Bone mineral/element content was then expressed as a percent of dry bone weight. The paper presents comparative mineral/element content data between F and S for various recovery times. The use of the rat model for further understanding microgravic osteopenia is discussed.

PO4(-)

Ca2(+)

Preface

This work was accomplished under Project 72311416, "Biomaterials and Kinematics," and was performed in the Biodynamic Effects Branch, Biodynamics and Bioengineering Division, Air Force Aerospace Medical Research Laboratory. The authors gratefully acknowledge the assistance of Mr. K. Sousa and Dr. E. Holten for their support of these experiments. This project is funded in part by NASA PR-A71669B. The authors also extend thanks to Pollution Control Sciences, Inc., Miamisburg, Ohio, for expertly performing all bone mineral/element content analysis under AF Contract No. F33615-81-C-0509.



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Introduction

Over the past ten years a vast amount of research pertaining to space flight induced osteopenia has been conducted. Attempts have been made to determine its etiology, the severity of its consequences and its reversibility upon return to earth's environment. These studies, incorporating biochemical, histological and biomechanical analysis techniques and various ground based experimental models to simulate the effects of space flight have centered mainly upon the cortical and cancellous regions of long bone to determine the bone dynamic change caused by weightlessness. Little, if any, of this work has concentrated on bone loss and skeletal readaptation within the vertebral column. Information pertaining to this skeletal region is important because of the association of reduced cancellous vertebral bone with decreased mechanical stress resistance within the vertebral column, the consequence of which are much more severe than fracture of appendages.

The mission of COSMOS 1129 was to study the adaptive abilities of organisms to microgravity followed by readaptation in earth's environment. One experiment of this project dealt with alteration in rat skeletal mineral dynamics during exposure to microgravity and earth's readaptation. This paper describes the methods and results of a comparative vertebral centrum bone mineral content analysis from ground based control and space flown rats.

Materials and Methods

Experimental Animal

The test subjects for this study were male Wistar rats. At the initiation of experimentation, the animals weighed between 270 to 320 grams and were approximately 85 days old. Three separate animal sets were exposed to different treatment schemes (Flight, Synchronous Control and Vivarium Control) in the following manner.

Flight (F): These rats were subjected to 18.5 days of orbital space flight aboard COSMOS 1129 biosatellite. Each animal was housed in a separate environmentally controlled chamber during launch, space flight and earth reentry. After space flight recovery the F rats were either sacrificed immediately (R+O) or allowed to readapt to earth's environment for 6 (R+6) or 29 (R+29) days before death.

Synchronous Control (S): In an effort to differentiate the effects of space flight exposure from those related to launch and reentry stresses, a ground based synchronous control experiment was conducted. For this treatment, an attempt was made to provide the rat with an environment as close as possible to that experienced by the F animals without microgravic exposure. The S rats were housed in containers similar to the F rats and received simulated launch and recovery mechanical stress separated by 19 days. After reentry stress exposure the S animals were sacrificed using the same schedule applied to the F treatment group (R+O, R+6 and R+29).

Vivarium Control (V): This treatment provided controls for both the F and S treatment groups. The V rats received minimal mechanical stress exposure and were provided with similar housing, food, water, lighting and climate as for F and S rats. The same schedule of readaptive duration (18.5 day control followed by either R+O, R+6 and R+29) applied to the other two treatment groups was utilized for the V rats.

For this project the vertebral columns of 24 animals (8 each R+O, R+6 and R+29) were obtained from each treatment group. At the time of sacrifice of

each rat, the entire vertebral column was excised, examined and stored in a frozen state to await biomechanical testing and bone mineral/element analysis.

Specimen Preparation and Biomechanical Testing

Rat vertebral centra were prepared for biomechanical compression testing by 1) disarticulation from the vertebral column via transection of the intervertebral disc and synovial articular facet capsules, 2) removal of the posture process at the pedicle and 3) elimination of adjoining soft tissue. The prepared specimens were then compression tested in an Instron Material Test Machine in a manner described in detail by Kazarian (1980). The compression tested centra were then frozen in distilled sterile H_2O to await mineral/element analysis.

A statistical analysis of the data obtained from the vertebral centra compression tests indicated no significant difference in the strength characteristics for V and S rats. It was therefore decided upon the basis of this fact and financial consideration to accomplish mineral/element analysis on F and S rats only.

Bone Mineral Analysis

In preparation for bone mineral/element analysis each compression tested vertebral sample was thawed, removed from its container and rinsed with distilled water (the rinse being saved within the original container). The rinsed specimens were dried, desiccated, and weighed to the nearest .01 mg. to obtain total specimen weight. Each weighed vertebral centrum along with its storage and rinse H_2O was digested with 10 ml of 10% nitric acid in a 50 ml volumetric flask. The flasks were brought to volume with distilled H_2O, and the analyte transferred to storage bottles and maintained in a cooler at 4° C to await analysis.

The minerals/elements analyzed for each vertebral specimen included Ca^{2+}, PO_4^-, K, Na, Ba, Sr, Cl^-, F^-, Mg, Pb, Mn, and Y. Atomic absorption spectrophotometry, specific Ion electrode measurement and colormetric analysis conducted in accordance with 40 CFR 136 were the three basic procedures utilized. The techniques employed along with a list of

minerals/elements analyzed are presented in Table I and briefly discussed below.

TABLE I

Bone Mineral/Element Analysis Procedures

Procedure	Mineral/Element
1. Atomic Absorption Spectrophotometry	
a) Air/Acetylene Flame	Ca^{2+}, Mg
b) Air/Propane Flame	Na, K
c) Oxide/Acetylene Flame	Ba, Sr, Y
d) Graphite Furnace	Pb, Mn
2. Specific Ion Electrode Measurement	F^-, Cl^-
3. Colormetric Analysis	PO_4^-

Atomic Absorption Analysis: All metal analyses were performed using an Instrumentation Laboratory Model 951 Atomic Absorption Spectrophotometer. To control chemical interferences and/or ionization, all analytical solution for Ca^{2+} and Mg contained .1% lanthanum and solutions for Ba, Sr, and Y received 2 mg/ml potassium. Dilutions were required for many of the analytic solutions prepared. Standards and blanks were analyzed approximately every three to five analyses to correct for any absorbance drift and to recalibrate the instrument.

Specific Ion Electrode: Orion fluoride and chloride electrodes were used in conjunction with a standard pH meter to analyze vertebral mineral content for Fl^- and Cl^- respectively. Standard sample curves were prepared and analyzed daily on each pH meter to insure accuracy.

Colorimetric Analysis: All phosphate analyses were performed colorimetrically using the preliminary digestion/Ascorbic Acid Method. Standard samples were prepared and analyzed daily to insure proper instrumentation calibration.

Because some of the mineral/elements analyzed were found to exist in very low concentration in the vertebral samples, the minimum detection limit for each questionable analytic was determined by analyzing 25 to 50 blanks (Table II). Also duplicate analyses were employed on a number of samples to determine the reproducibility of the data.

TABLE II

Limits of Detection

Analyte	Limit of Detection	
	mg/l	wt %*
Lead	.024	.0050
Manganese	.014	.0029
Barium	.012	.0025
Strontium	.012	.0252
Fluoride	.2	.0420
Chloride	.5	.1049
Yttrium	.5	.1049

*Based on average centrum dry weight of 23.48 mg

Data Analysis

To facilitate data analysis and comparison, the vertebral centra were grouped into six equal positions according to descending column level. (P_1=T_2-T_3-T_4, P_2=T_5-T_6-T_7...P_6=L_5-L_6-L_7). This grouping was based upon similar properties and size of adjacent vertebral centra.

Means and standard deviations were determined for each exposure group (F and S), at each vertebral position (P_1 through P_6) and for each recovery period (R+O, R+6 and R+29). The students T statistical analysis of the difference of means was used to determine significant differences ($p=.05$) between exposure groups for different recovery periods at each column position.

Results

Seven of the total mineral/elements analyzed were found unacceptable for further consideration. The reasons for their rejection were either that the majority of weight % were at or below the detection limits of the analysis method (Cl^-, F^-, Mn, Pb and Y) or contaminates from blood or marrow trapped within the specimens produced unacceptable data error (Na, K). Vertebral centra mineral/element content findings for Sr, Ba, Mg, PO_4^- and Ca^{2+} are presented graphically in Figures 1 through 5 and briefly described below. Only trends and variations validated as statistically significant are reported below.

Strontium: The vertebral body content of Sr ranged from 0 to .2% weight. The statistical analysis of the Sr wt % data indicated there were no significant differences between any F recovery group (R+O, R+6 and R+29), between any S recovery group and between F and S treatments at most column levels. The few exceptions to the above observations showed no trends.

Barium: The mineral content of Ba ranged from .1 to 1% weight. Statistically, this data indicated that for the S treatment group, the Ba content decreased with increasing recovery duration for all column positions. For the F treatment, the Ba wt % increased above R+O levels for R+6 and returned to R+O level at R+29 for the lower column positions (P_4-P_6). Due to the extreme variations in the S group data, a comparison of S vs F treatment at any

recovery period yielded uninterruptable results.

Magnesium: Mg content analysis indicated wt. % levels ranging from .3 to .5. Statistically, for the S treatment there were no significant differences between any recovery periods at almost all column positions. For the F treatment, there was a significant increase in Mg content for R+0 compared to R+6 or R+29 at all column positions. No differences existed between F/R+6 and F/R+29. A comparison of F and S treatment/recovery values showed an increase of vertebral centrum Mg content at F/R+0 returning to S levels at F/R+6 and F/R+29 for all column positions.

Phosphate: PO_4^- content ranged from 9 to 13 % wt. No statistically significant differences were demonstrated for any combinations of treatment/recovery groups (S/R+0, S/R+6, S/R+29, F/R+0, F/R+6 and F/R+29).

Calcium: Ca^{+2} vertebral centrum content ranged from 19 to 24 wt. %. A trend not apparent in other mineral analyses was a decreasing Ca^{2+} content with decreasing column level for all treatment/recovery combinations except F/R+6 and S/R+6. Statistically, there was no significant differences between recovery periods within either treatment group, but there was a significant decrease in Ca^{2+} content when comparing F with S at each recovery period. This effect was most pronounced in the lower column position (P_3-P_6) for R+0, in the upper column position (P_1-P_4) for R+6, and for all column positions for R+29.

Discussion

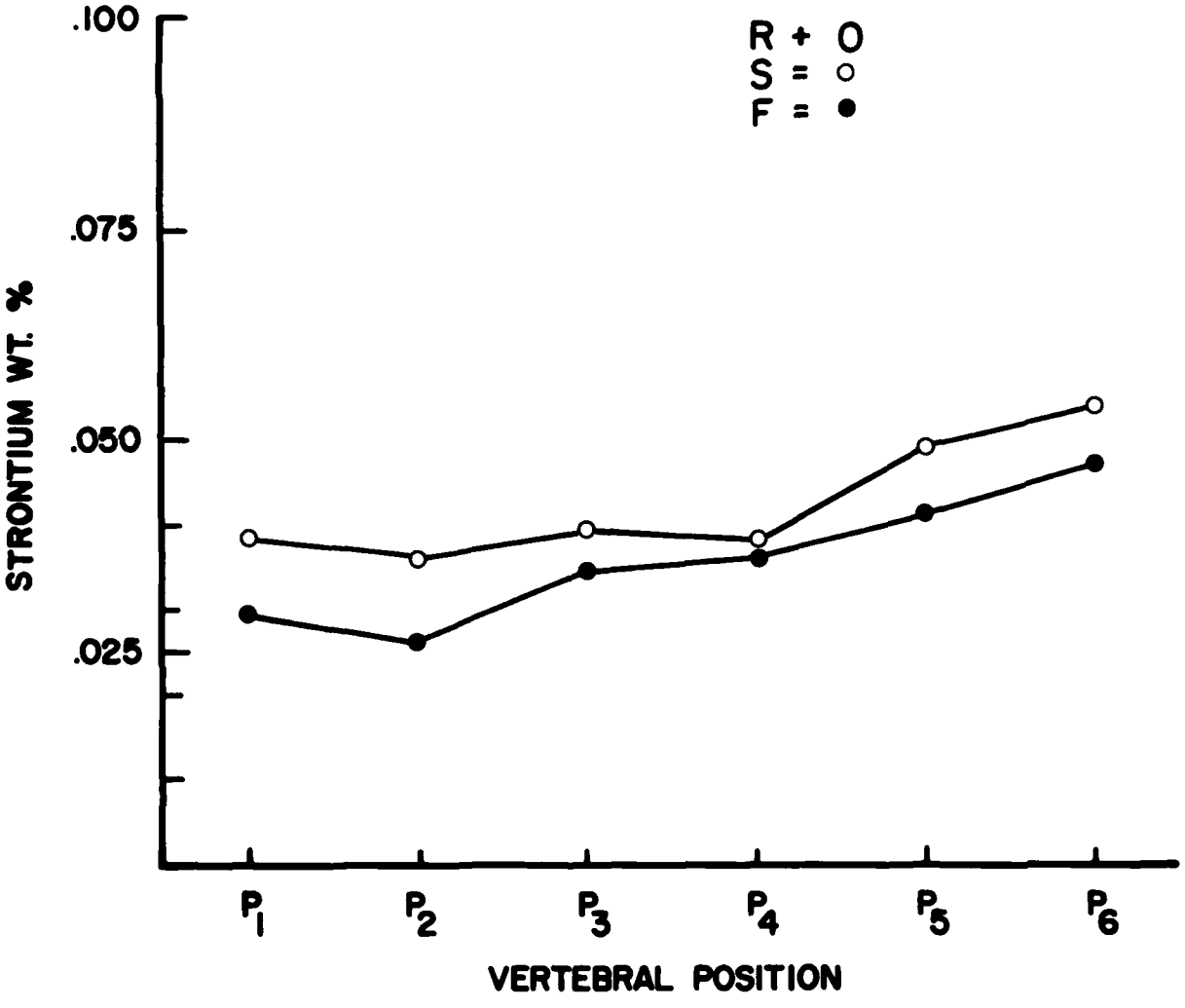
The purpose of this paper is to present results from a selective mineral/element analysis of F and S rat vertebral centra, and no attempt will be made to explain these results with specific skeletal physiologic conclusions. However, a selective review of literature upon this subject was found to be useful in explaining some of the results in general. Because no definitive studies pertaining to Mg skeletal metabolism are available, the observation of an increase Mg content for space flown rat cannot be explained. Further research is required to classify this observation. The results of PO_4^- content analysis did not seem to correspond with the results for Ca^{2+}.

There was no concomitant loss of Phosphate and Calcium as would be expected for decreased formation or increased resorption of bone. This observation may be the result of phosphate contamination from cell and marrow not removed before mineral content analysis and/or the result of a selection osteoid mineralization. Neuman (1981) has provided information suggesting that the calcium to phosphorous ratio varies with the maturation of newly formed ostroid. The techniques and analysis method utilized in this study were not selective enough to validate or disprove either explanation. The results of the calcium content analysis seem to support the observations of Wronski, et al. (1981) pertaining to F rat bone formation and resorption for cortical bone. Their conclusions indicated that space flight exposure caused an arrest in bone formation with no change in resorption rate. The curves for Ca^{2+} for F animal are decreased from S levels almost equally for all recovery groups. These results may indicate that similar mineral dynamics are functioning for both cortical and cancellous weight bearing bone.

It is tempting with such results, to attempt to extrapolate these findings to higher order animals. It was found, however, that almost no correlation seems to exist between the findings of hypokinetic and space borne experimentaion for rat and other experimental animals. These results may be explained by the findings of Klein (1981). Using Ca^{45} to study steady-state relationships between bone and blood in dogs, chicks, and rats, he found that bone formation was disassociated early (2 weeks of life) from bone resorption in the rat. He concluded that the rat is not an appropriate model for studying active bone turnover. The results of this study seem to indicate a similar conclusion.

Figures 1-15. Rat vertebral centrum weight % St, Ba, Mg, PO_4^-, Ca for Flight (F) and Synchronous (S) exposure at recovery periods 0(R+6) and 29(R+26) days.

Figure 1



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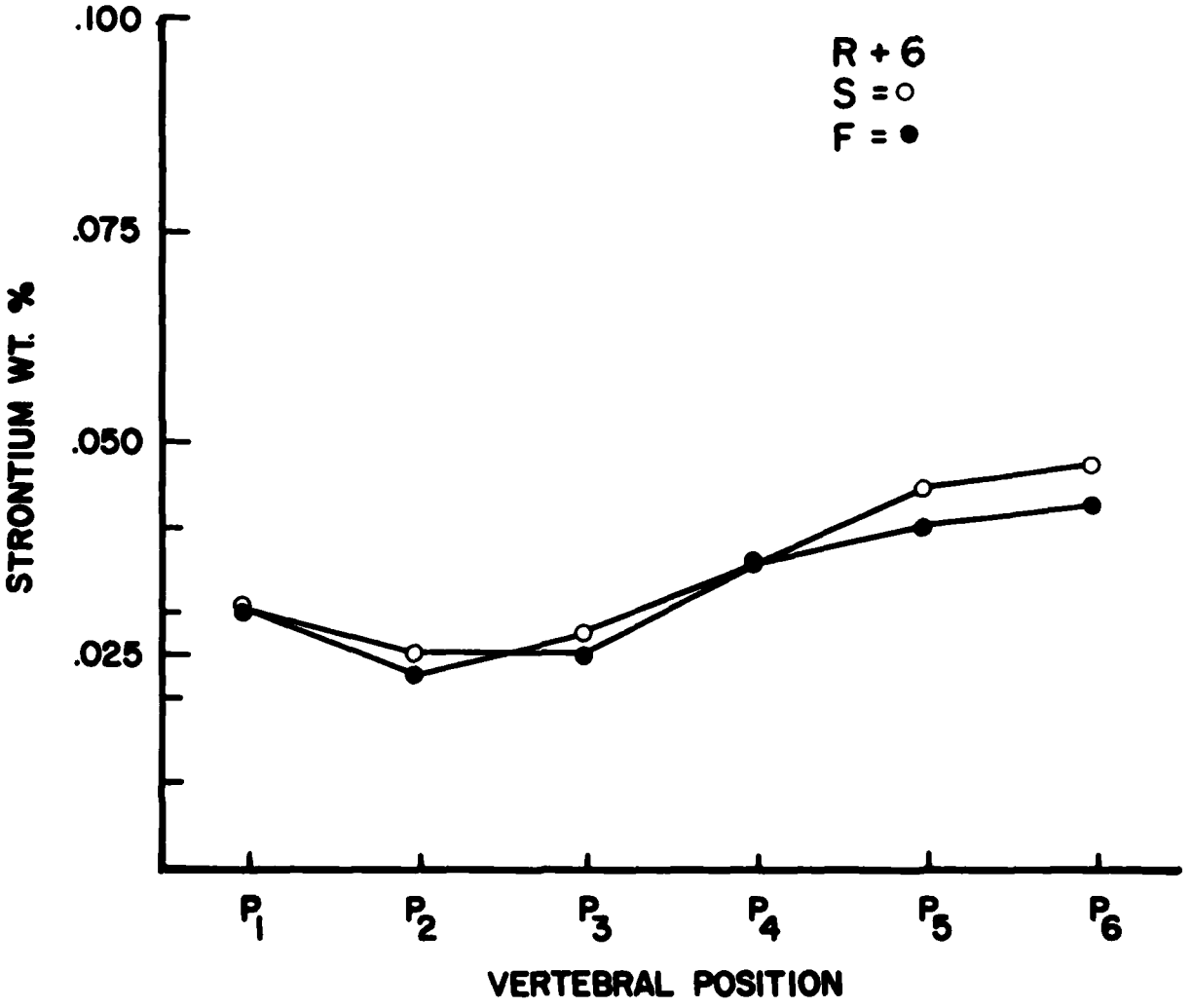
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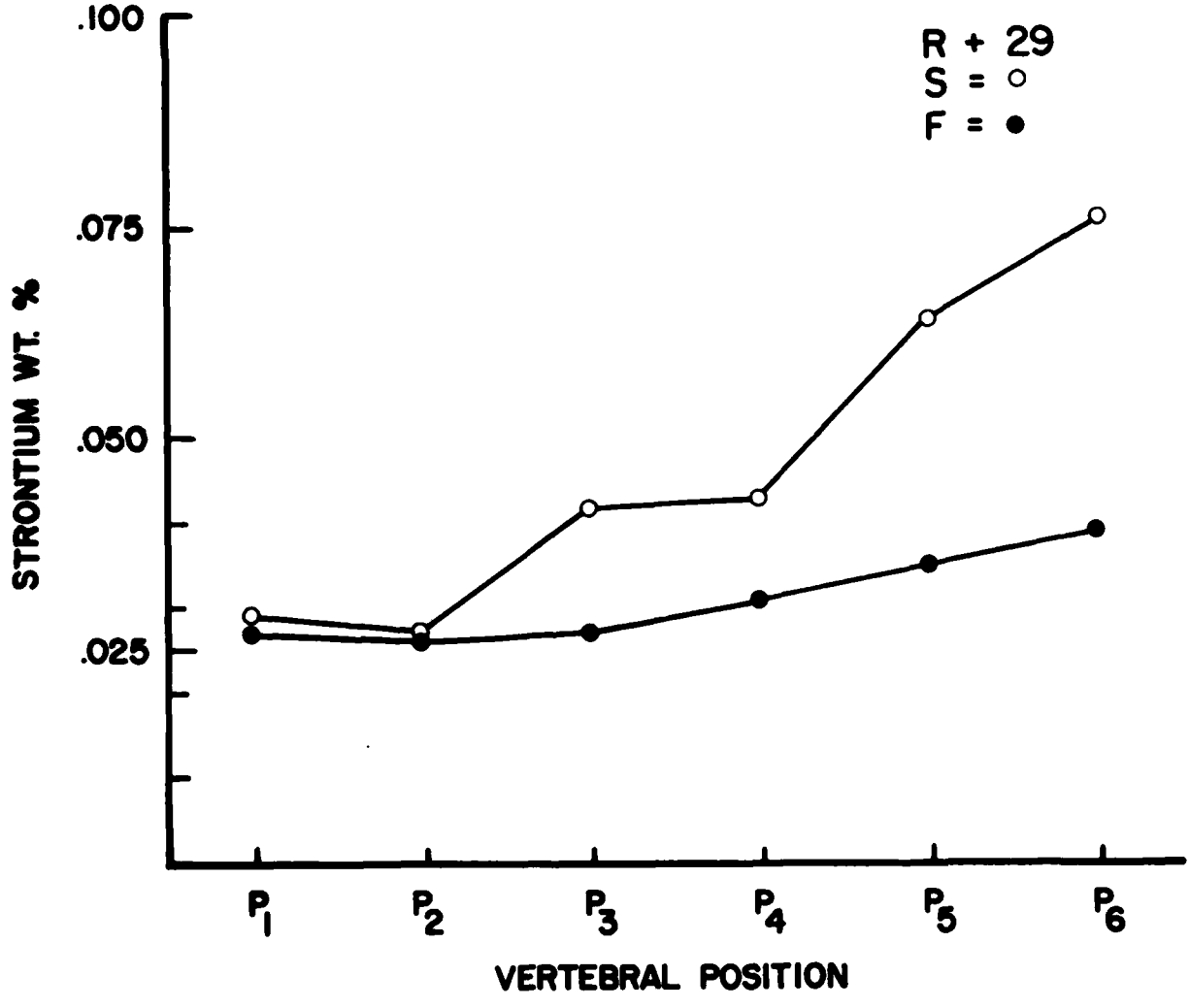
.075
.050
.025
STRONTIUM WT. %
R + 6
S = ○
F = ●
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 3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.025
STRONTIUM WT. %
R + 29
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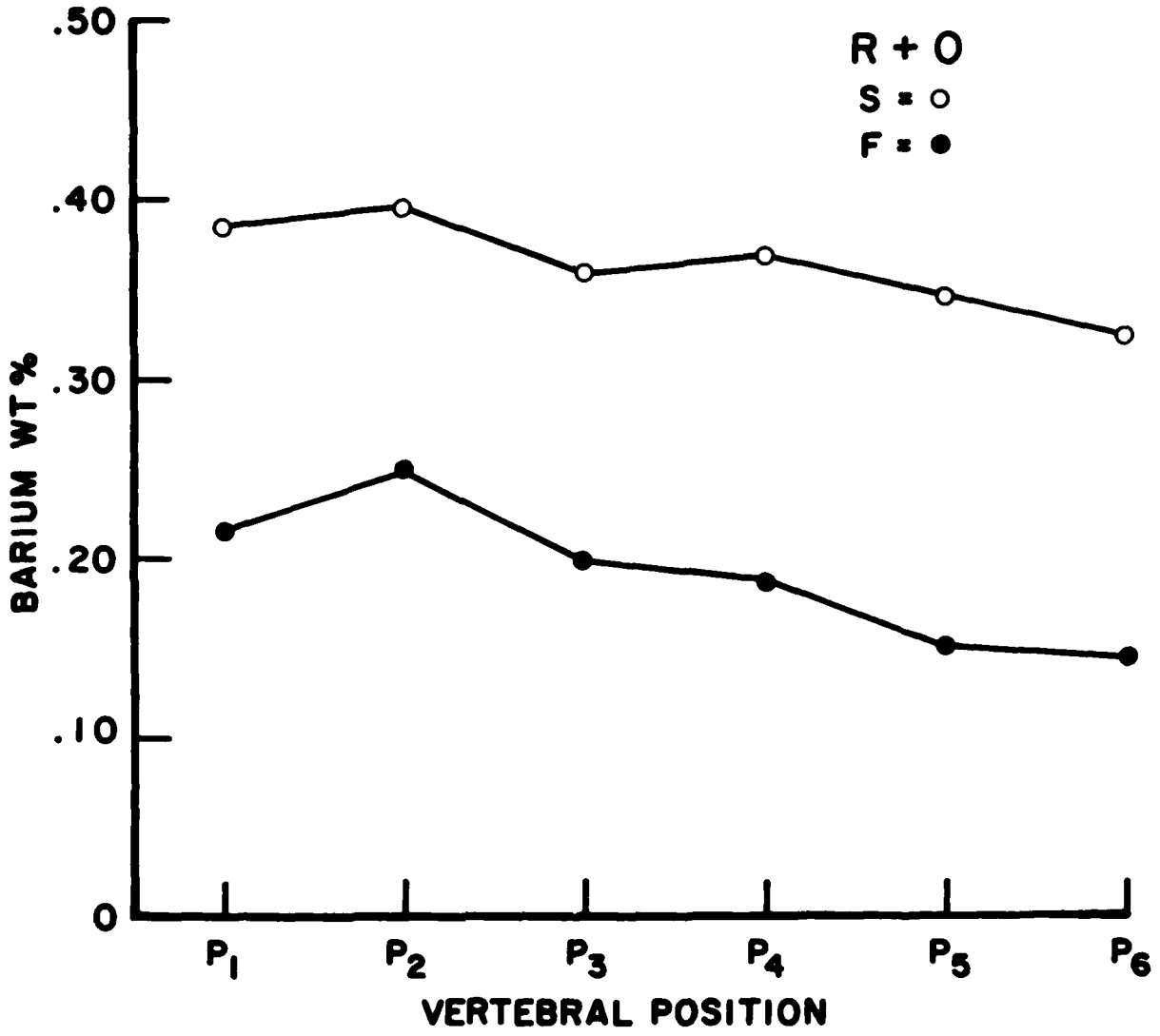
F = ●
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 4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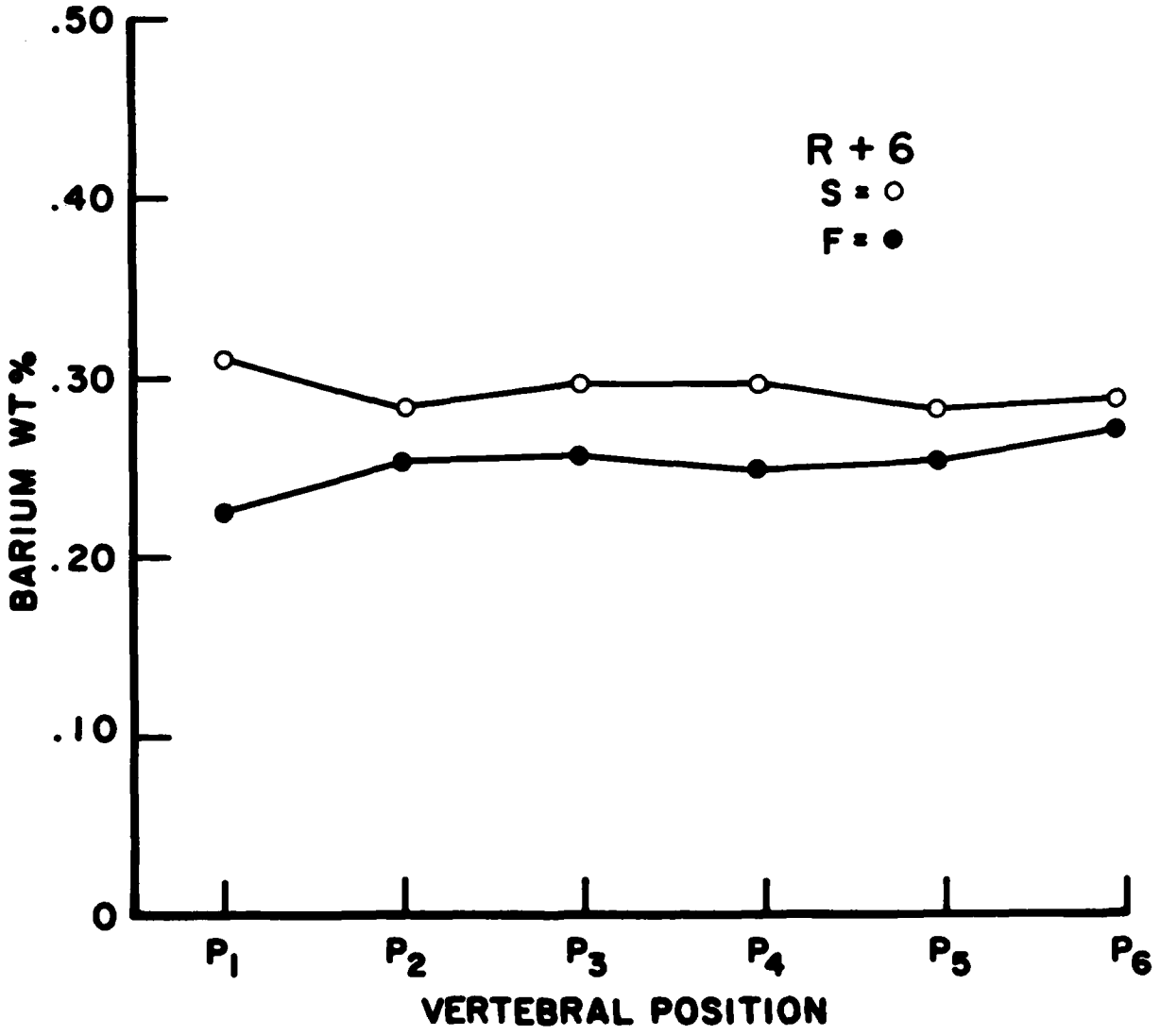
.30
.20
.10
0
BARIUM WT %
R + O
S = ○
F = ●
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 5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BARIUM WT %
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F = ●
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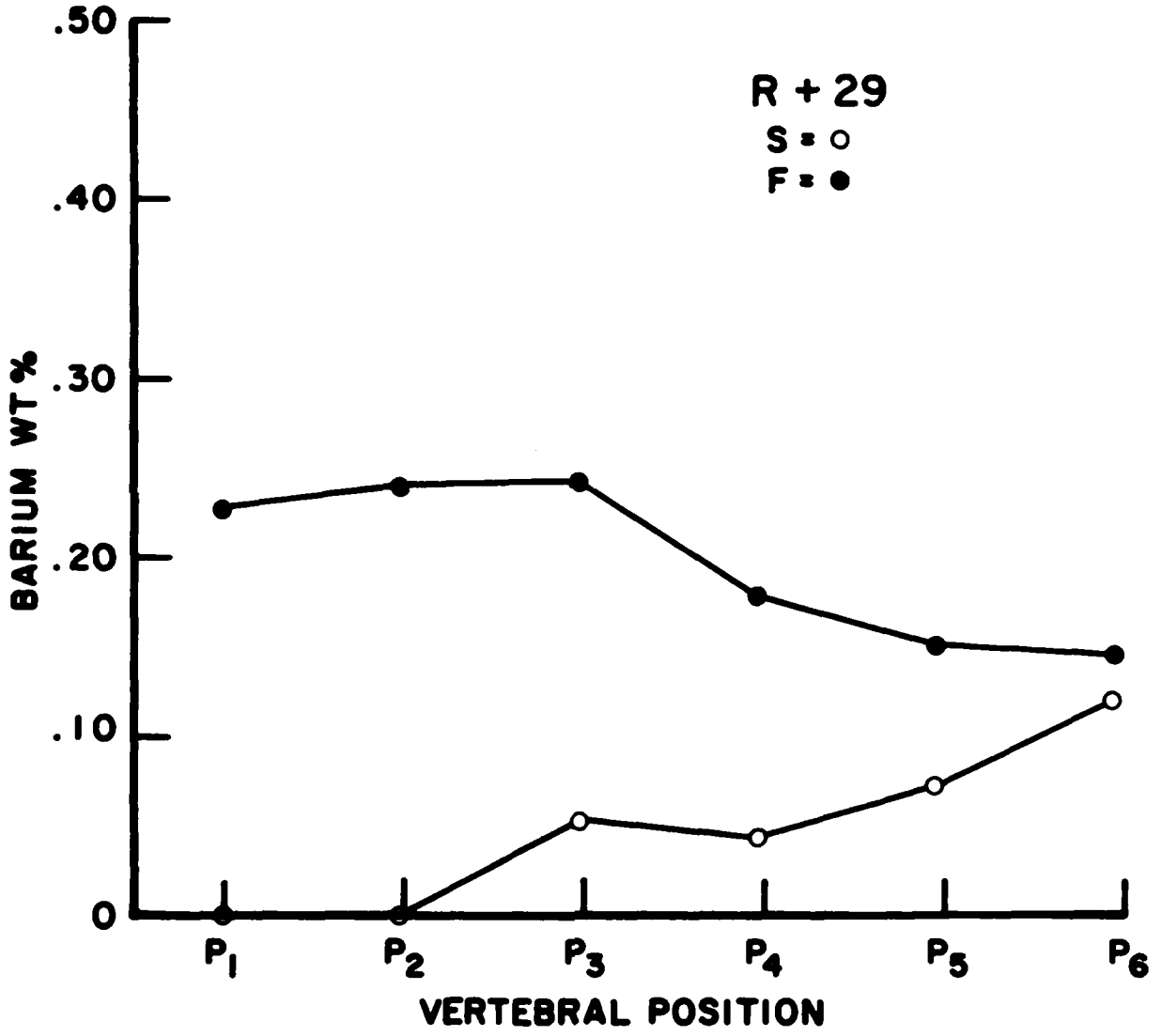
P6
VERTEBRAL POSITION

Figure 6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BARIUM WT %
R + 29
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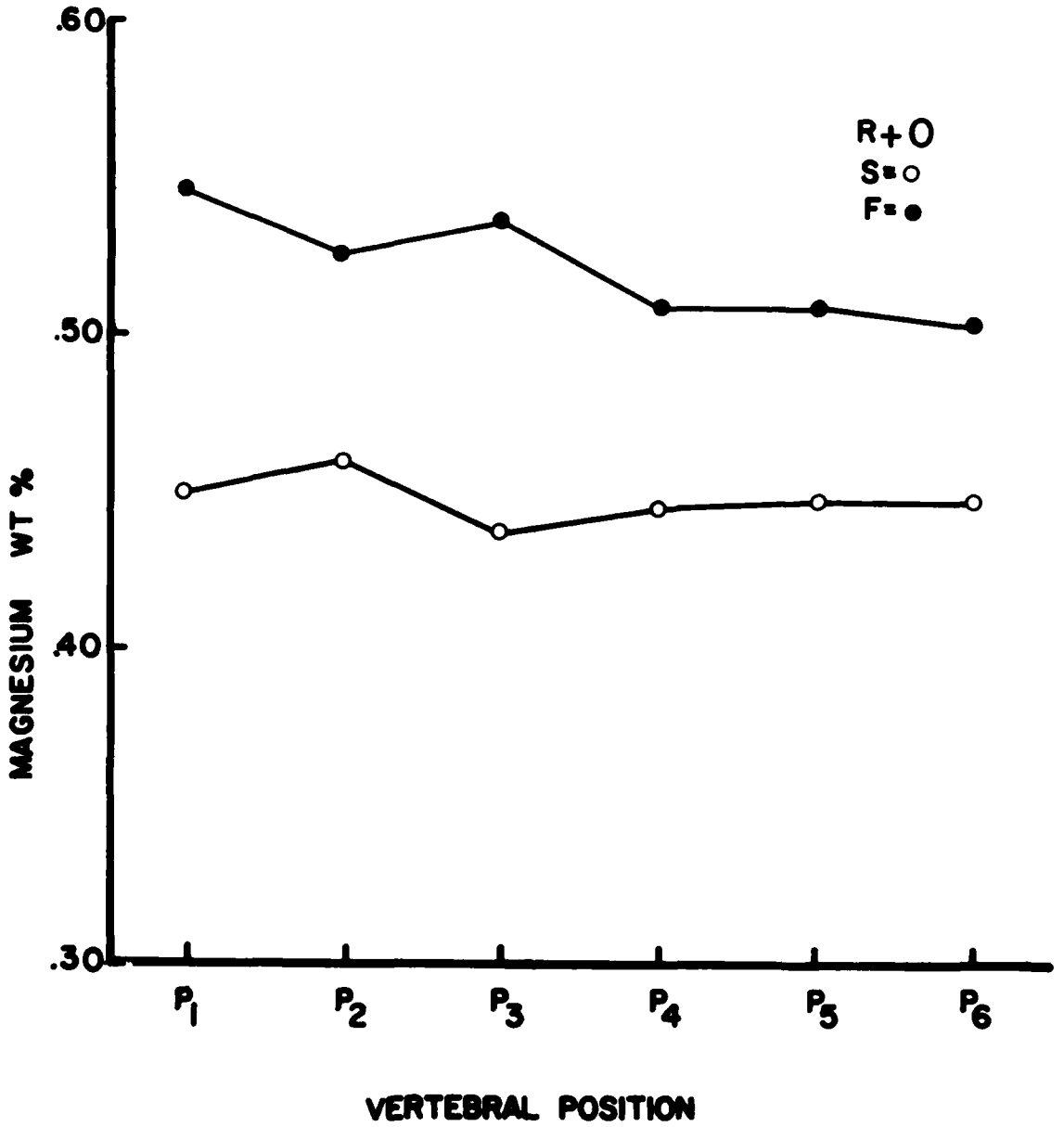
F = ●
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 7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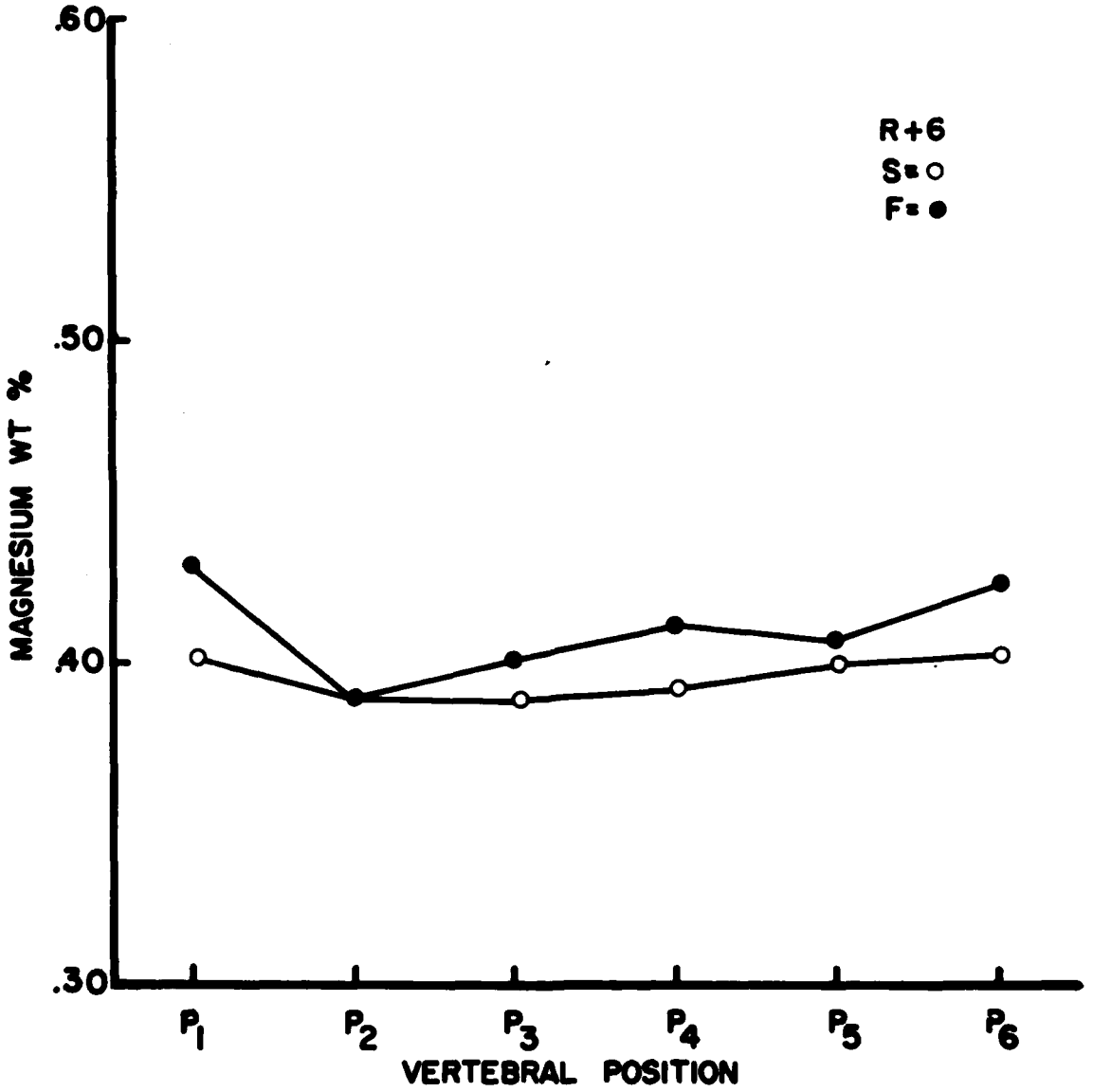
R+O
S=O
F=●
MAGNESIUM WT %
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 8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MAGNESIUM WT %
R+6
S=○
F=●
P1
P2
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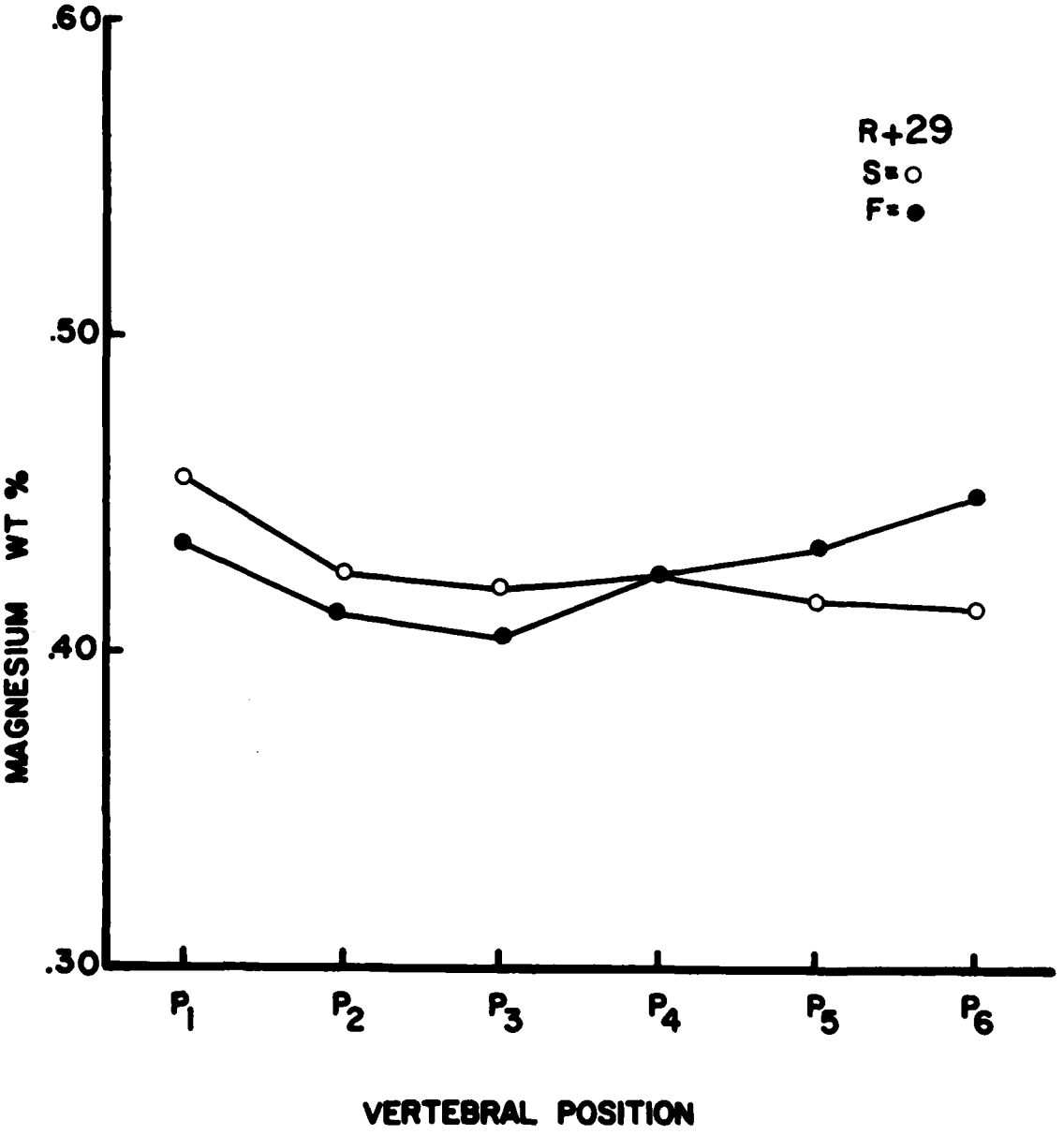
P4
P5
P6
VERTEBRAL POSITION

Figure 9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F=●
MAGNESIUM WT %
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P2
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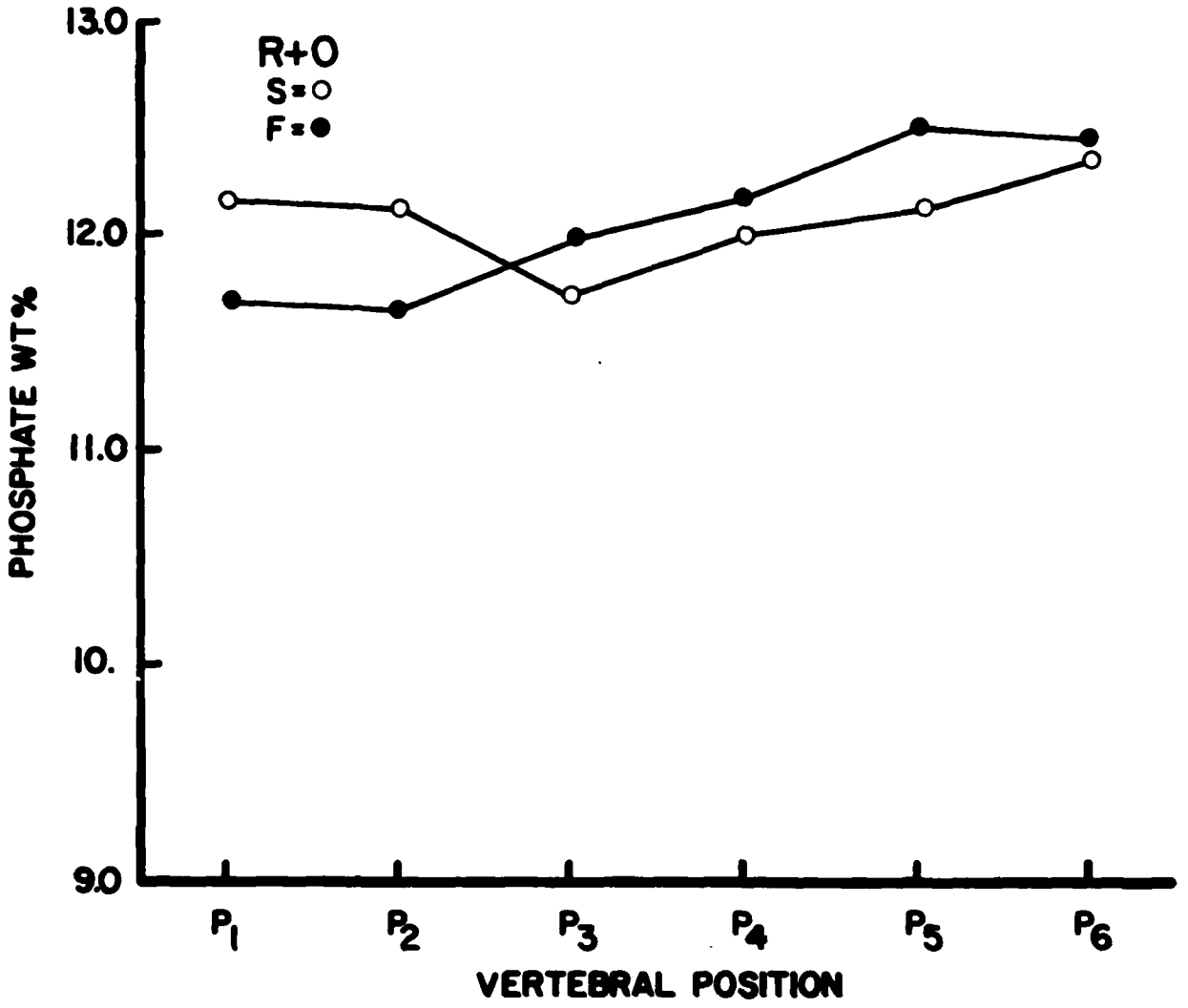
P4
P5
P6
VERTEBRAL POSITION

Figure 10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10.
9.0
R+0
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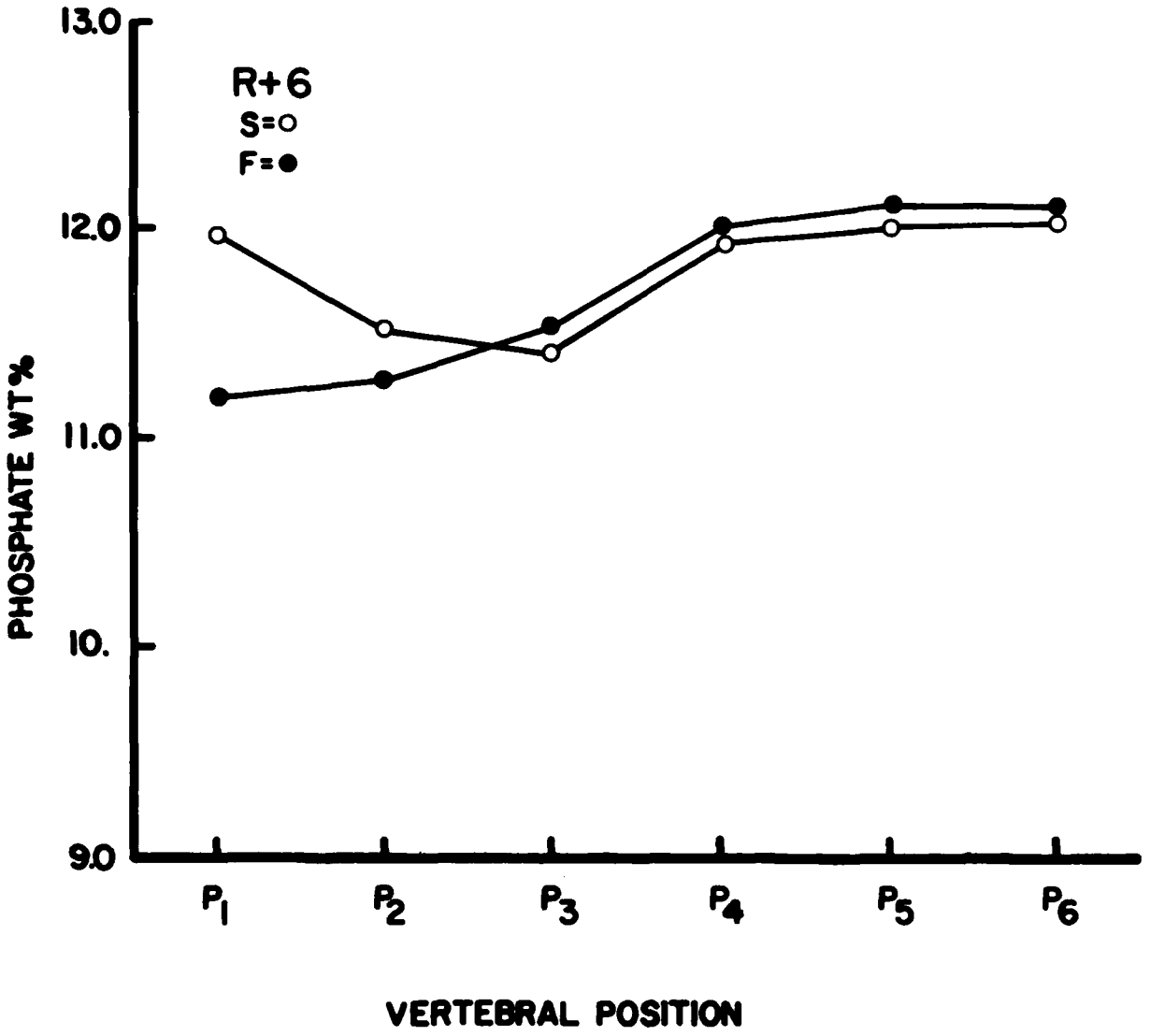
F=●
PHOSPHATE WT%
P1
P2
P3
P4
P5
P6
VERTEBRAL POSITION

Figure 11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PHOSPHATE WT%
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P2
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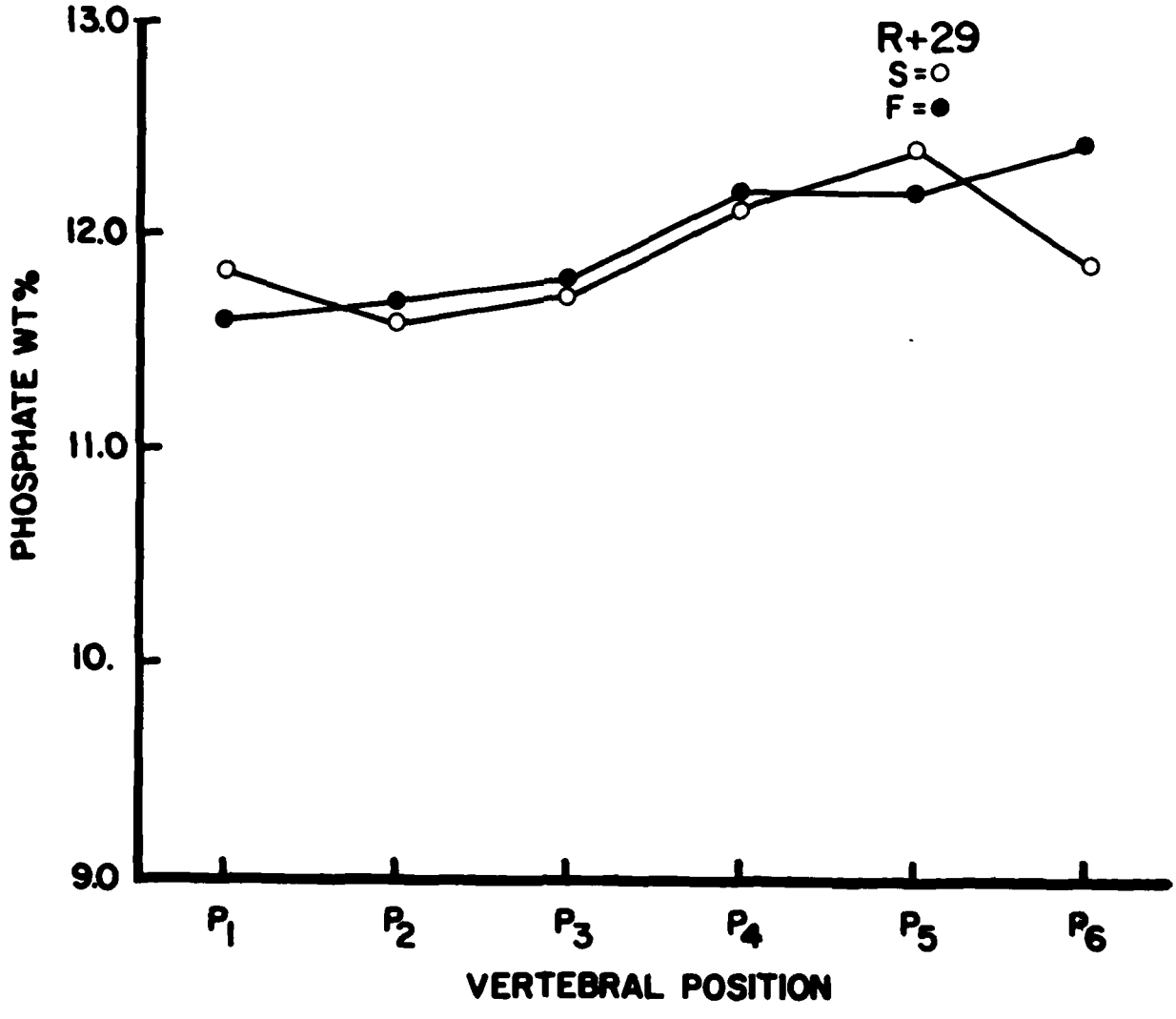
P5
P6
VERTEBRAL POSITION

Figure 12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R+29
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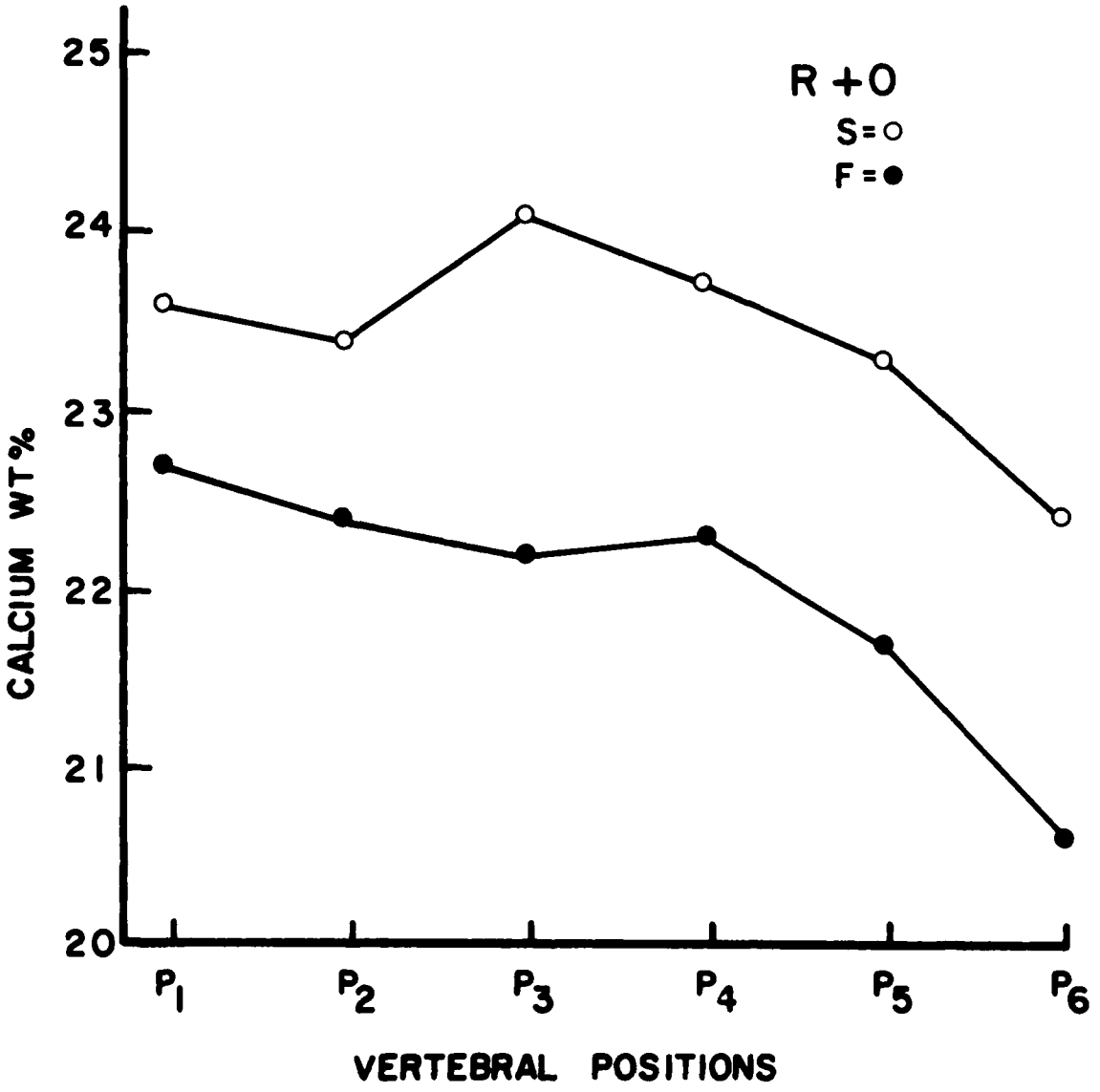
P6
VERTEBRAL POSITION

Figure 13


R +O
S=○
F=●
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CALCIUM WT%
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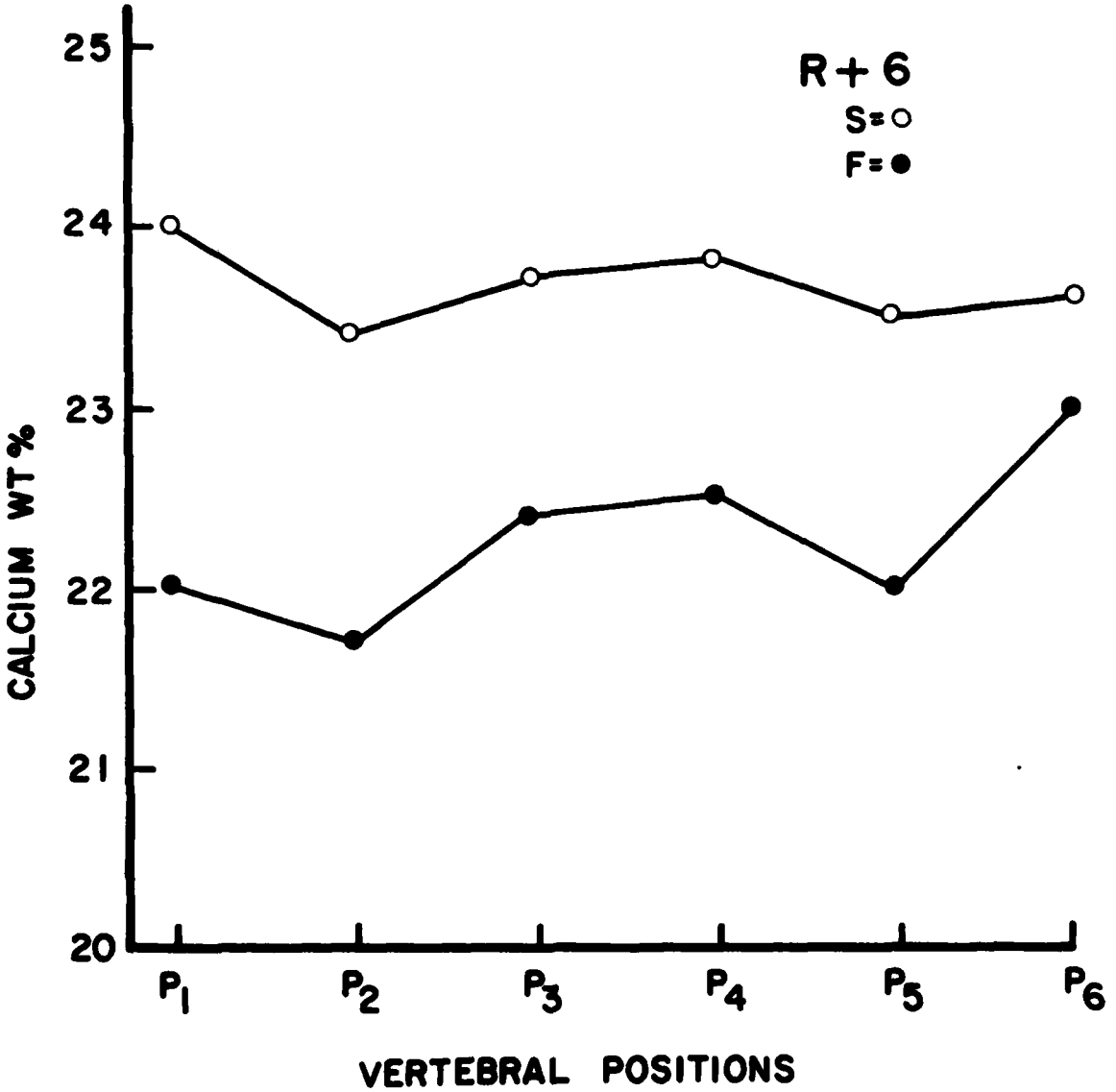
P6
VERTEBRAL POSITIONS

Figure 14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CALCIUM WT %
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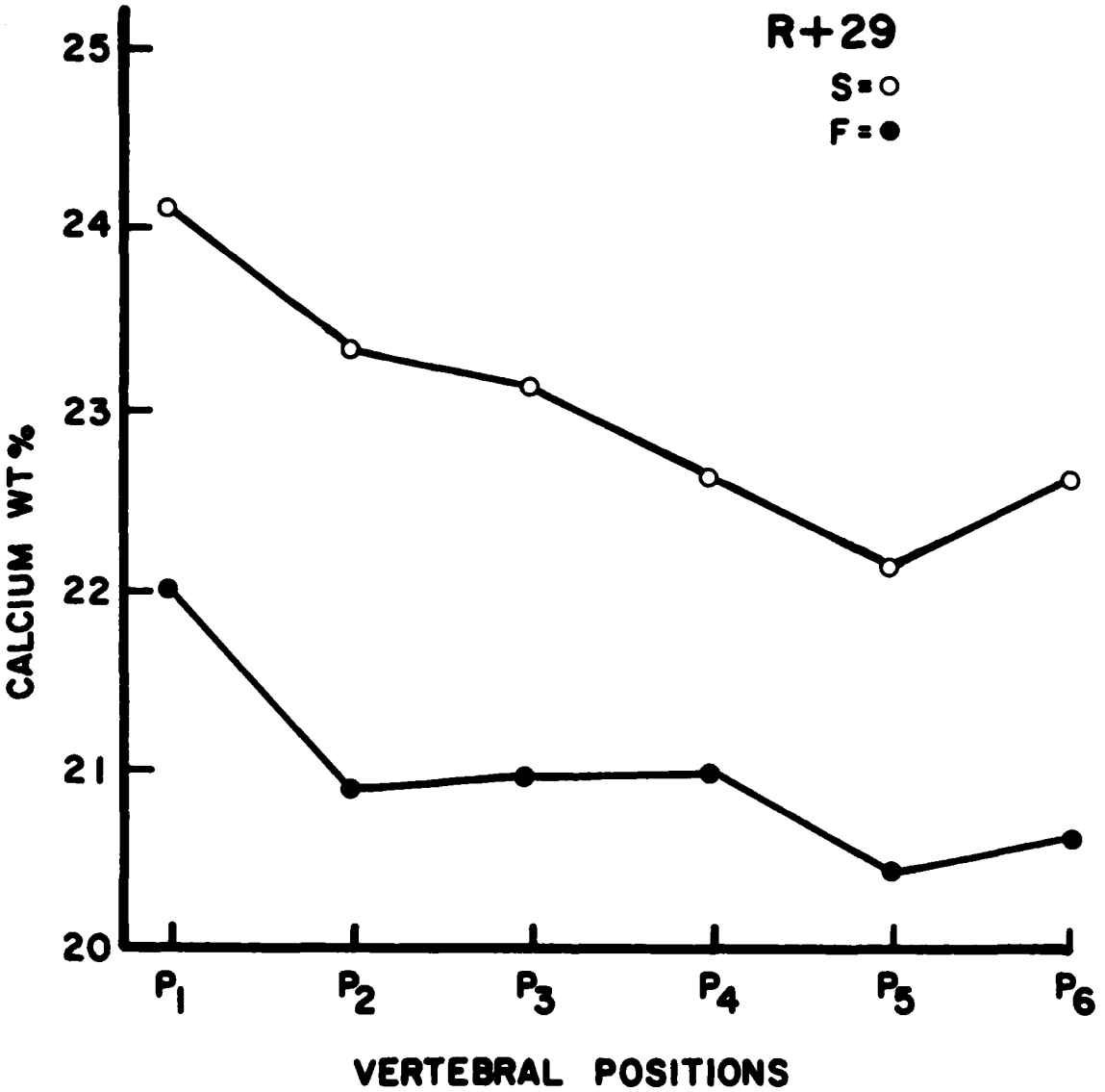
P6
VERTEBRAL POSITIONS

Figure 15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CALCIUM WT %
P1
P2
P3
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VERTEBRAL POSITIONS

Summary

The conclusions of the study pertaining to microgravic induced rat vertebral centra mineral/element content variations are as follows:

Comparing the microgravically exposed (F) and Control (S) rats vertebral centra mineral/element content -

- There was no significant difference in strontium content.
- There was a slight increase in magnesium content at 0 days recovery returning to control level for 6 and 29 days recovery.
- There was no significant difference in phosphate content.
- There was a significant decrease in calcium content for all recovery times.

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